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Association Between Early-Onset Alcoholism and the Dopamine D2 Receptor Gene

Yoshihiro Kono,[1]* Hiroshi Yoneda,[1] Toshiaki Sakai,[1] Yasuhiro Nonomura,[1] Yasuhiro Inayama,[1] Jun Koh,[1] Jun Sakai,[1] Yasushi Inada,[1] Hiroyuki Imamichi,[2] and Hiroyuki Asaba[1]

[1]*Department of Neuropsychiatry, Osaka Medical College, Takatsuki, Osaka, Japan*

[2]*Shin-Abuyama Hospital, Takatsuki, Osaka, Japan*

We examined the allelic association between the dopamine D2 receptor (DRD2) gene and alcoholism in 100 biologically unrelated Japanese alcoholics and 93 unrelated controls. Genomic DNA was prepared from peripheral white blood cells using the phenol-chloroform method. A 310-bp region surrounding the TaqA site at the DRD2 locus was amplified by polymerase chain reaction (PCR), and the PCR product was incubated with *Taq*I. The A1 allele remained intact while the A2 allele was cut. The frequency of the A1/A1 genotype and the frequency of the A1 allele were higher in early-onset alcoholics than in controls, $P < 0.05$ and $P < 0.01$, respectively. Moreover, the frequency of the A1/A1 genotype and the frequency of the A1 allele were higher in early-onset alcoholics with family histories of alcohol dependence than in controls, $P < 0.01$ and $P < 0.01$, respectively. The results indicate that the DRD2 gene is associated with susceptibility to early-onset alcoholism, and that each additional A1 allele shifts onset of alcoholism to an earlier age. Am. J. Med. Genet. 74:179–182, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: association; alcoholism; DRD2 gene; PCR; RFLP

INTRODUCTION

Family, twin, and adoption studies have shown that genetic factors play an important role in the etiology of alcoholism [Goodwin, 1979; Cloninger et al., 1981; Devor and Cloninger, 1989]. There is evidence that alcohol stimulates brain reward systems in part through its action on central dopaminergic nervous systems [Koob and Bloom, 1988]. Such neurobiological findings raised the question of whether the structure or expression of genes of dopaminergic neurotransmission could contribute to vulnerability to alcoholism. Bunzow et al. [1988] cloned rat dopamine D2 receptor complementary DNA. Moreover, Grandy et al. [1989] mapped the human D2 receptor gene to the q22–q23 region of chromosome 11, and identified a *Taq*I restriction fragment length polymorphism (RFLP). Using this RFLP analysis, Blum et al. [1990] found an allelic association between the DRD2 gene and alcoholism. To confirm their result, we studied the allelic association of the DRD2 gene with alcoholism in Japanese alcoholics.

SUBJECTS AND METHODS

Subjects

We examined 100 (78 males and 22 females) biologically unrelated alcoholics and 93 (70 males and 23 females) unrelated controls. They were all native Japanese living in the western area of Japan. Alcoholics and individuals with family histories of alcoholism were excluded from the controls. The mean ages ± SD of the alcoholics and controls were 48.7 ± 9.4 and 42.5 ± 13.3 years, respectively. Alcoholics were diagnosed as having alcohol dependence according to DSM-III-R by two psychiatrists independently without knowing the results of DNA typing. All alcoholics and controls gave informed consent.

The alcoholics were subtyped according to age of onset and family histories of alcohol dependence. Early-onset alcoholics were defined as those who were diagnosed as being alcohol-dependent according to DSM-III-R prior to age 25 years. Alcoholics were also subtyped according to whether they had, in addition, mood disorder, alcohol hallucinosis, alcohol withdrawal delirium, or alcohol-related seizures.

Methods

Genomic DNA was extracted from a 20-ml sample of heparinized peripheral venous blood using the phenol-

*Correspondence to: Yoshihiro Kono, Department of Neuropsychiatry, Osaka Medical College, Daigaku-cho 2-7, Takatsuki, Osaka 569, Japan.

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TABLE I. Genotypes and Allele Frequencies at DRD2

	Number of subjects	Genotypes			Allele frequencies	
		A1/A1	A1/A2	A2/A2	A1	A2
Alcoholics	100	19	40	41	0.395	0.605
Males	78	15	32	31	0.397	0.603
Females	22	4	8	10	0.364	0.636
Controls	93	10	49	34	0.371	0.629
Males	70	6	40	24	0.371	0.629
Females	23	4	9	10	0.370	0.630

chloroform method. We used the method reported by Grandy et al. [1993] for PCR detection of the TaqA RFLP at the DRD2 locus. A 310-bp region (Genbank accession no. L22303) surrounding the TaqA site at the DRD2 locus was amplified by PCR. PCR was carried out with the primers 971:5′-CCGTCGACG-GCTGGCCAAGTTGTCTA-3′ and 5014:5′-CCGTCGACCCTTCCTGAGTGTCATCA-3′. The PCR reaction was carried out in a 50-μl volume, containing 200 ng genomic DNA, 50 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP, and 2.5 U Taq DNA polymerase (Pharmacia Biotech, Uppsala, Sweden). After an initial 5-min denaturation at 94°C, the reaction mix was processed by use of a Perkin Elmer thermocycler (model 2400-R, Perkin Elmer, CA) according to the following program: 35 cycles of 60 sec at 94°C, 60 sec at 50°C, and 60 sec at 72°C, with final extension for 5 min at 72°C.

Ten μl of the PCR product were digested with five units of *Taq*I at 65°C. Electrophoresis of the digest was carried out with 3% agarose gel, and the bands were visualized by ethidium bromide staining. The A1 allele remained intact, while the A2 allele was cut into 130-bp and 180-bp pieces.

Distribution of age at onset was examined to determine whether or not age of onset was related to the A1 allele dose.

RESULTS

Genotypes and allele frequencies at DRD2 of the alcoholics and controls did not significantly differ from those expected from the Hardy-Weinberg equilibrium (Tables I–III). The frequencies of the A1 allele at DRD2 were 0.395 in alcoholics, and 0.371 in controls. Ten (11%) of the 93 controls and 19 (19%) of the 100 alcoholics were homozygous for the A1 allele (Table I). There were no significant differences in the frequencies of the A1 allele and the A1/A1 genotype at the DRD2 locus between the alcoholics and controls. However, in the 21 early-onset alcoholics, the A1 allele frequency was 0.452 and 6 (29%) of those 21 early-onset alcoholics were homozygous for the A1 allele (Table II). The frequency of the A1/A1 genotype and the frequency of the A1 allele were higher in the early-onset alcoholics than in the controls, $P < 0.05$ and $P < 0.01$, respectively.

In the 12 early-onset alcoholics with family histories of alcohol dependence, the A1 allele frequency was 0.583, and 5 (42%) of those 12 alcoholics were homozygous for the A1 allele (Table II). The frequency of the A1/A1 genotype and the frequency of the A1 allele were higher in the early-onset alcoholics with alcoholic family histories than in the controls, $P < 0.01$ and $P < 0.01$, respectively.

The frequencies of the A1 allele and the A1/A1 genotype of the alcoholics, divided into four subgroups, are given in Table III: 20 alcoholics had mood disorder; 11, alcohol hallucinosis; 29, alcohol withdrawal delirium; and 10, alcohol-related seizures. The A1 allele frequencies were 0.375, 0.409, 0.328, and 0.400, respectively. The A1/A1 genotype was found in 3 (15%) alcoholics with mood disorder, in 2 (18%) with alcohol hallucinosis, in 3 (10%) with alcohol withdrawal delirium, and in 2 (20%) with alcohol-related seizures. The frequencies of the A1 allele and the A1/A1 genotype showed no differences between the alcoholics with mood disorder, alcohol hallucinosis, alcohol withdrawal delirium, or alcohol-related seizures, and the controls.

Gene dose effect of the A1 allele showed that each additional A1 allele shifted onset to an earlier age. As shown in Figure 1, at age 40 years, 68.4% of alcoholics with two A1 alleles, 57.5% of alcoholics with one A1 allele, and 46.3% of alcoholics without any A1 allele were diagnosed as alcoholics. The mean ages of onset were 35.3 ± 10.3, 38.5 ± 10.2, and 41.6 ± 12.3 years, respectively.

TABLE II. Genotypes and Allele Frequencies at DRD2

	Number of subjects	Genotype			Allele frequencies	
		A1/A1	A1/A2	A2/A2	A1	A2
Early-onset alcoholics	21	6*	7	8	0.452**	0.548
Alcoholics with family histories of alcohol dependence	35	8	14	13	0.429	0.571
Early-onset alcoholics with family histories of alcohol dependence	12	5**	4	3	0.583**	0.417
Controls	93	10	49	34	0.371	0.629

*$P < 0.05$.
**$P < 0.01$.

TABLE III. Genotypes and Allele Frequencies at DRD2

	Number of subjects	Genotypes			Allele frequencies	
		A1/A1	A1/A2	A2/A2	A1	A2
Alcoholics with mood disorder	20	3	9	8	0.375	0.625
Alcoholics with alcohol hallucinosis	11	2	5	4	0.409	0.591
Alcoholics with alcohol withdrawal delirium	29	3	13	13	0.328	0.672
Alcoholics with alcohol-related seizures	10	2	4	4	0.400	0.600
Controls	93	10	49	34	0.371	0.629

DISCUSSION

Blum et al. [1990] examined *Taq*I RFLP to the dopamine D2 receptor gene in deceased alcoholics, and found a positive association between the A1 allele of DRD2 and alcoholism. However, subsequent studies by other investigators failed to confirm this. Bolos et al. [1990] found no significant difference in the prevalence of the A1 allele at DRD2 between alcoholics and controls. They reported that the A1 allele and alcoholism were neither linked nor cosegregated in two pedigrees. Gelernter et al. [1991] and Turner et al. [1992] found no allelic association between the A1 allele at DRD2 and alcoholism. On the other hand, Comings et al. [1991] reported that the A1 allele of the DRD2 gene is associated with a number of behavior disorders such as alcoholism, Tourette's syndrome, and attention deficit hyperactivity disorder; they speculated that the A1 allele may act as a modifying gene rather than as the primary etiological agent of these behavior disorders.

An association between alcoholism and the dopamine D2 receptor gene remains controversial; however, there may be an association between severe alcoholism and the A1 allele at DRD2 [Cloninger, 1991; Conneally, 1991; Noble et al., 1991; Parsian et al., 1991; Uhl et al., 1992; Arinami et al., 1993].

Noble et al., [1991] showed a decreased number of binding sites of D2 dopamine receptors in the brains of subjects in the order of A2/A2, A1/A2, and A1/A1 genotypes. Arinami et al. [1993] showed that order of severity is increased in the order of A2/A2, A1/A2, and A1/A1 genotypes.

Our study showed that the A1 allele of DRD2 was associated with early-onset alcoholism, with an age of onset prior to 25 years. Gene dose effect of the A1 allele showed that each additional A1 allele shifted onset to an earlier age.

These findings indicate that the DRD2 gene is associated with susceptibility to early-onset alcoholism, and that the A1 allele of DRD2 has a promotive effect on the onset of alcoholism.

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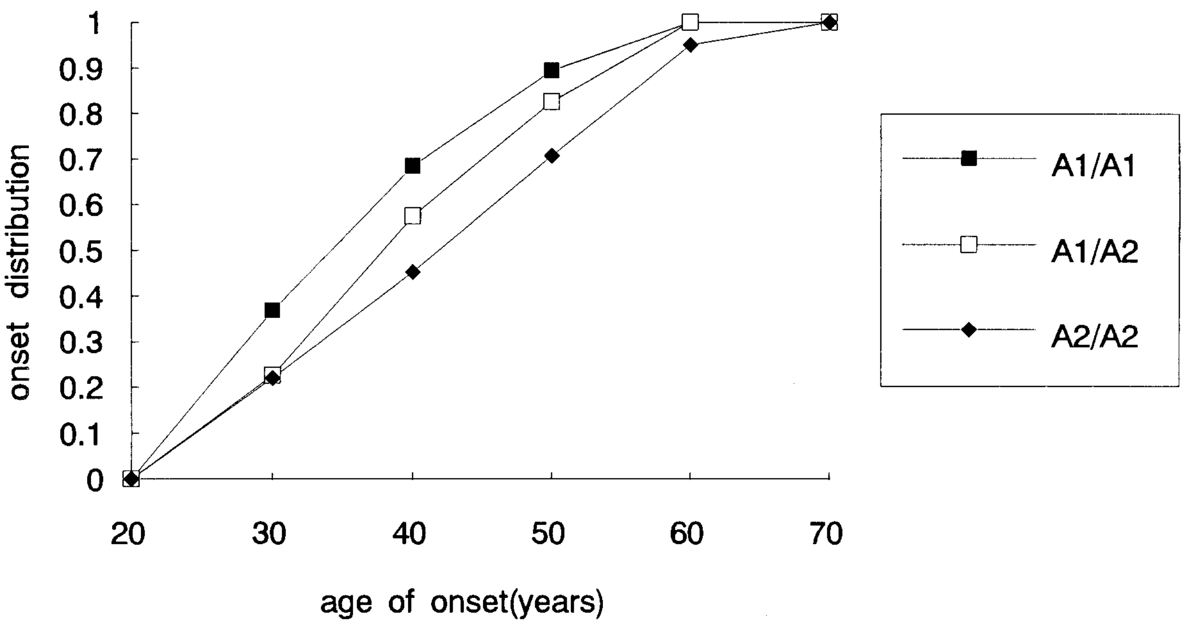

Fig. 1. Age at onset of alcoholics with 0, 1, and 2 A1 alleles.

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